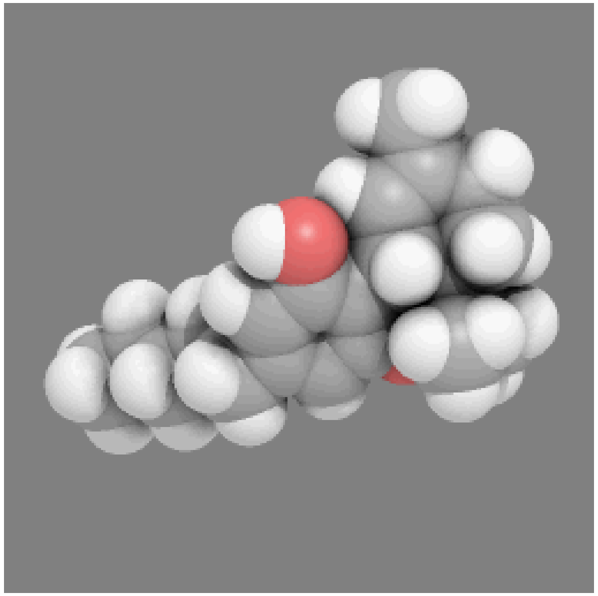
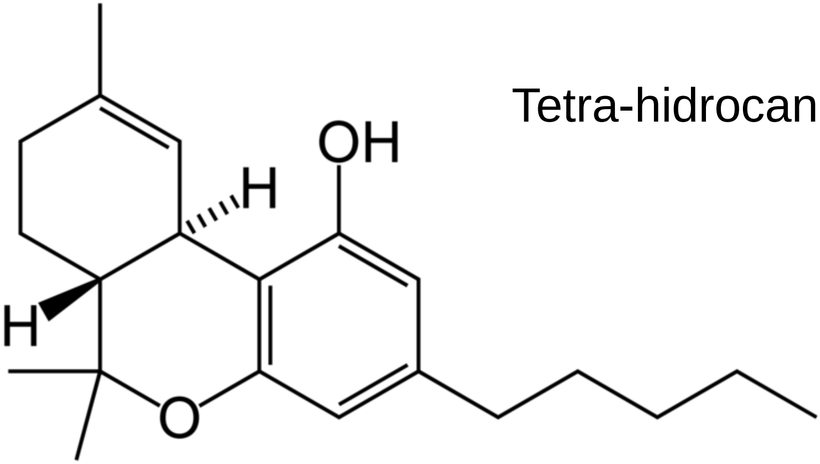
Grande parte dos Fármacos foi obtida, ou desenvolvida, a partir de produtos naturais

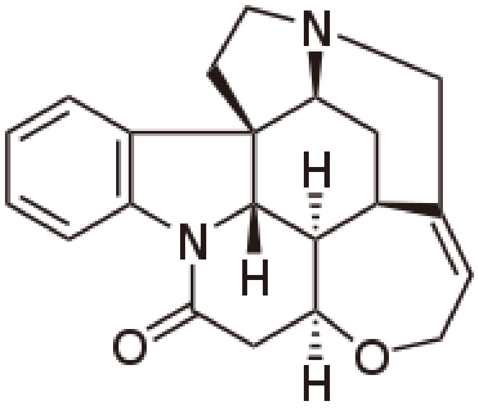




Tetra-hidrocanabinol

History

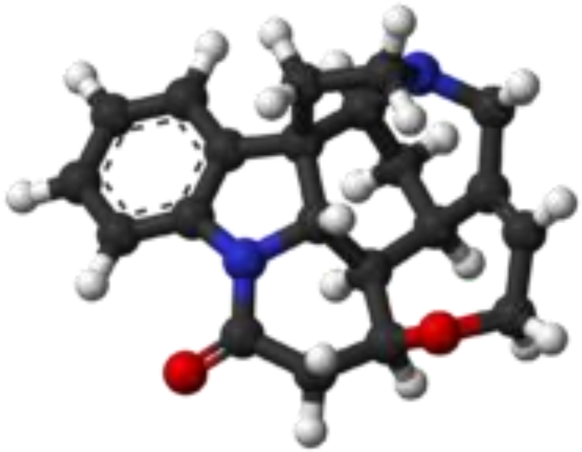
Structural elucidation of natural products used to be *very hard* and *take forever*.



Strychnine *alkaloid toxin*

Isolated by Pelletier & Caventou (1818)

Past: H. Leuchs worked
on structure for 40 years
until R. Woodward (1954) beat
him to it.



Today: <1 mg sample
needed; a weekend would
be enough.

Etapas para elucidação estrutural

- Determinação da Fórmula Molecular
- Caracterizar Grupos funcionais
- Degradação da Molécula e Síntese de Derivados

Atualmente

- Métodos Espectroscópicos

STRUCTURAL ELUCIDATION

- ***Spectroscopic methods:***

➢ ***Infrared (IR)***

- indicates presence of functional groups:

C=O ~ 1670 – 1750 cm^{-1}

amide, ketone, ester

OH, NH/NH2 ~ 3100 cm^{-1} to 3500 cm^{-1}

Limitation; non polar and semi polar compounds only.

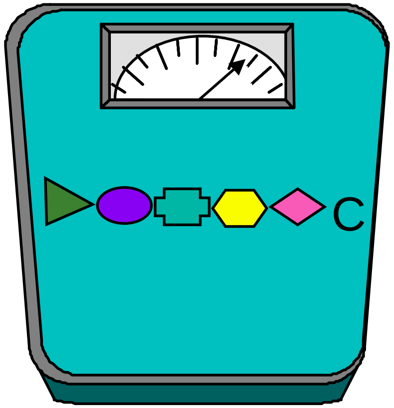
STRUCTURAL ELUCIDATION cont'd

- ***Mass Spectrometry***
- **Enables the determination of molecular weight.**
- **Aids structural elucidation – fragmentation peaks: loss of CO (M^+- 28), loss of H_2O (M^+- 18).**
- **Enables identification of mixtures; MS-MS.**
- **Various ionization techniques – to accommodate different compounds; polar, ionic, non-polar, macromolecules.**
- **Various Analyzers; usage (MS-MS, HRMS), cost.**

NUCLEAR MAGNETIC RESONANCE

- ***Nuclear Magnetic resonance:***
  - **Permits the establishment of the structural skeleton of the compound investigated.**
  - **^{1}HNMR showed resonances of protons while ^{13}C NMR showed the C resonances.**
  - **Allows to establish the connectivity between carbons and protons.**
  - **One dimensional and two dimensional techniques available:**
    - **COSY, HMQC, HMBC, NOESY etc.**
  - **For ^{1}HNMR ~ 1-5 mg (pure) sufficient**
  - **For ^{13}C NMR ~ 20 mg sufficient.**

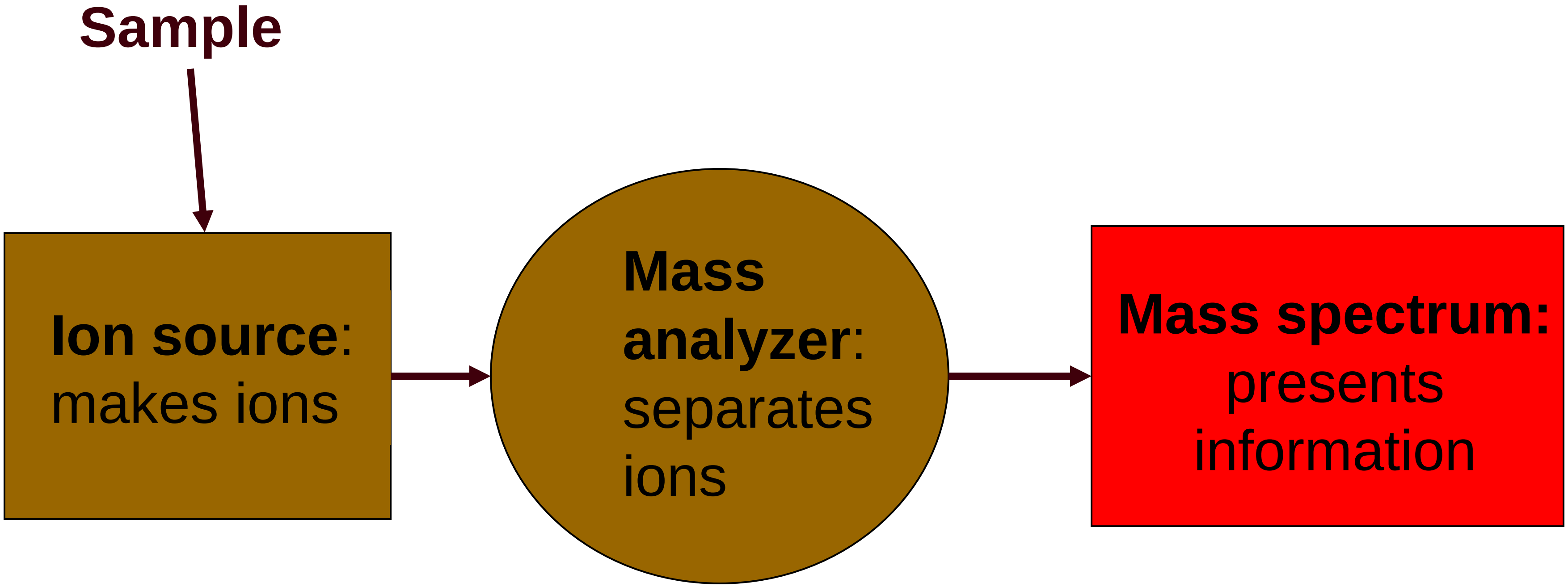
Espectrometria de Massas



Applications of Mass Spectrometry

- Pharmaceutical analysis
  - Bioavailability studies
  - Drug metabolism studies, pharmacokinetics
  - Characterization of potential drugs
  - Drug degradation product analysis
  - Screening of drug candidates
  - Identifying drug targets
- Biomolecule characterization
  - Proteins and peptides
  - Oligonucleotides
- Environmental analysis
  - Pesticides on foods
  - Soil and groundwater contamination
- Forensic analysis/clinical

How does a mass spectrometer work?



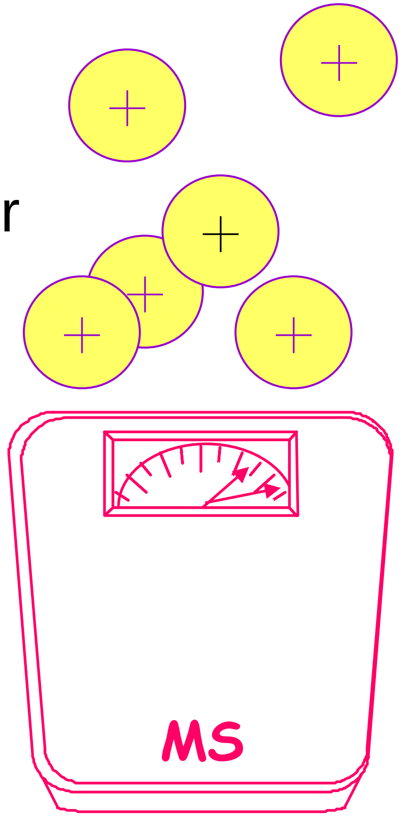
Sample

Ion source: makes ions

Mass analyzer: separates ions

Mass spectrum: presents information

- Only gaseous ions can be detected by MS:
  - MS provides molecular weight or fragmentations for structural information
  - according to their ***mass to charge ratio***
  - output signals : ***the relative abundance of each ionic species*****.**



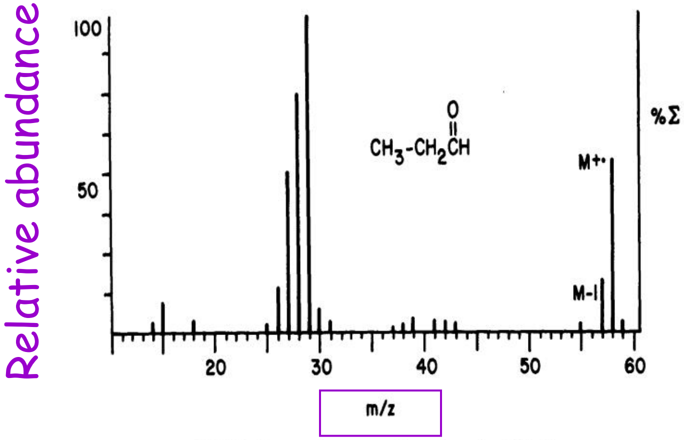
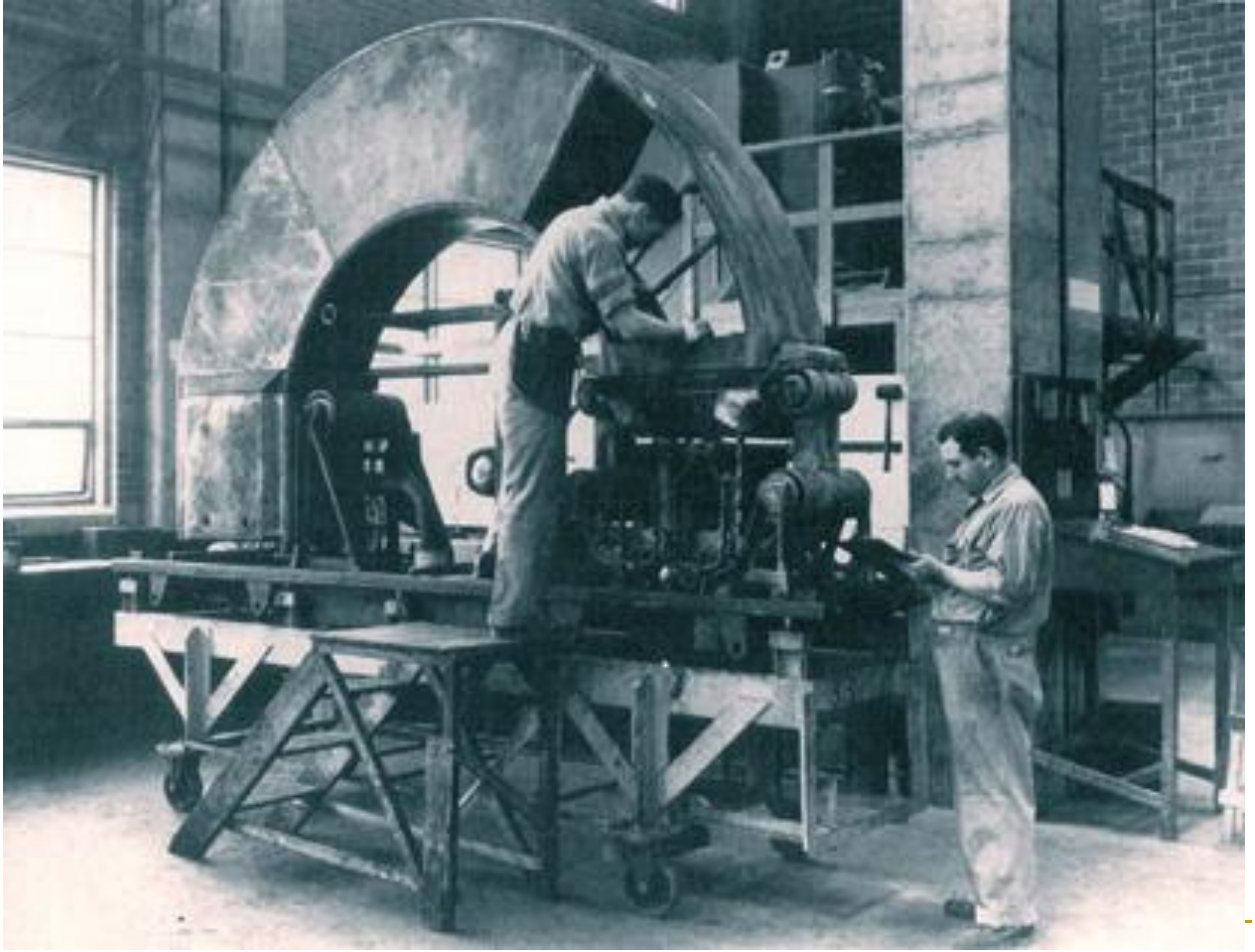


FIG. 1.7. Bar-graph mass spectrum of propionaldehyde.



http://masspec.scripps.edu/MSHistory/histpers.php


AutoSpec Ultima NT™
Pesticides
PCBs
P
PHARMACEUTICAL
CLINICAL
ENVIRONMENTAL
ANALYTICAL
Diox
IonSpec
Ultima FTMS

“Bench-top” LC/MS Systems

Mass Spectrometry Basics

What information can be determined?

- Molecular weight
- Molecular formula (HRMS)
- Structure (from fragmentation fingerprint)
- Isotopic incorporation / distribution
- Protein sequence (MS-MS)

Mass Spectrometry Basics

Mass spectrometry has 4 basic operations:

- Sample introduction (analyte must be in vapor phase)
- Ionization
- Mass analysis (separating ions by mass/charge ratio)
- Detection and quantitation

How to ionize neutral sample?

Positive ion mode

- Remove an electron → $M^{+\cdot}$
- Add one or more protons → $(M+nH)^{n+}$
- Fragmentation to produce ionized fragments,” fragment ions”

Ionization Methods

1. Electron Ionization (EI)

   most common ionization technique, limited to relatively low MW compounds (<600 amu)

2. Chemical Ionization (CI)

   ionization with very little fragmentation, still for low MW compounds (<800 amu)

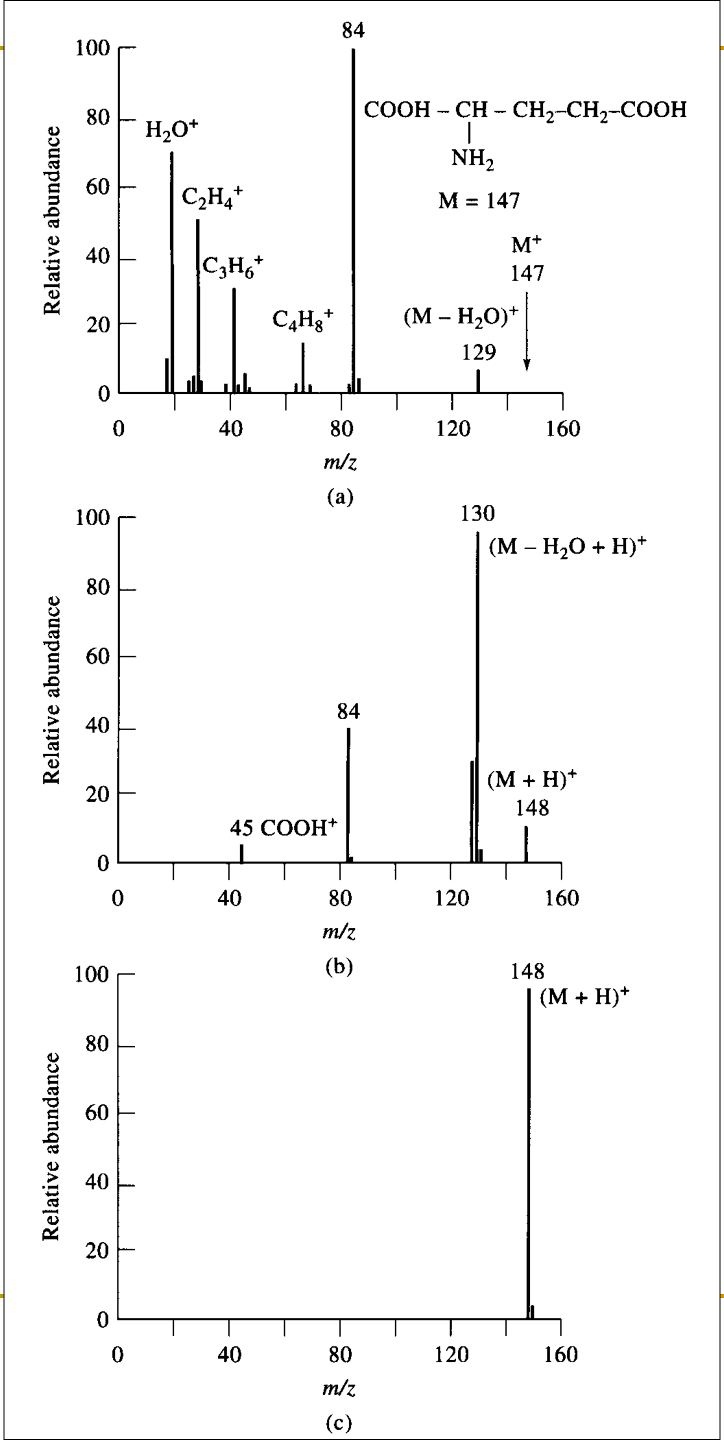
3. Desorption Ionization (DI)

   for higher MW or very labile compounds

4. Spray ionization (SI)

   for LC-MS, biomolecules, etc.

Glutamic Acid



Electron Impact (EI)

Field Ionization

Field Desorption

Electron Impact

(low picomole)

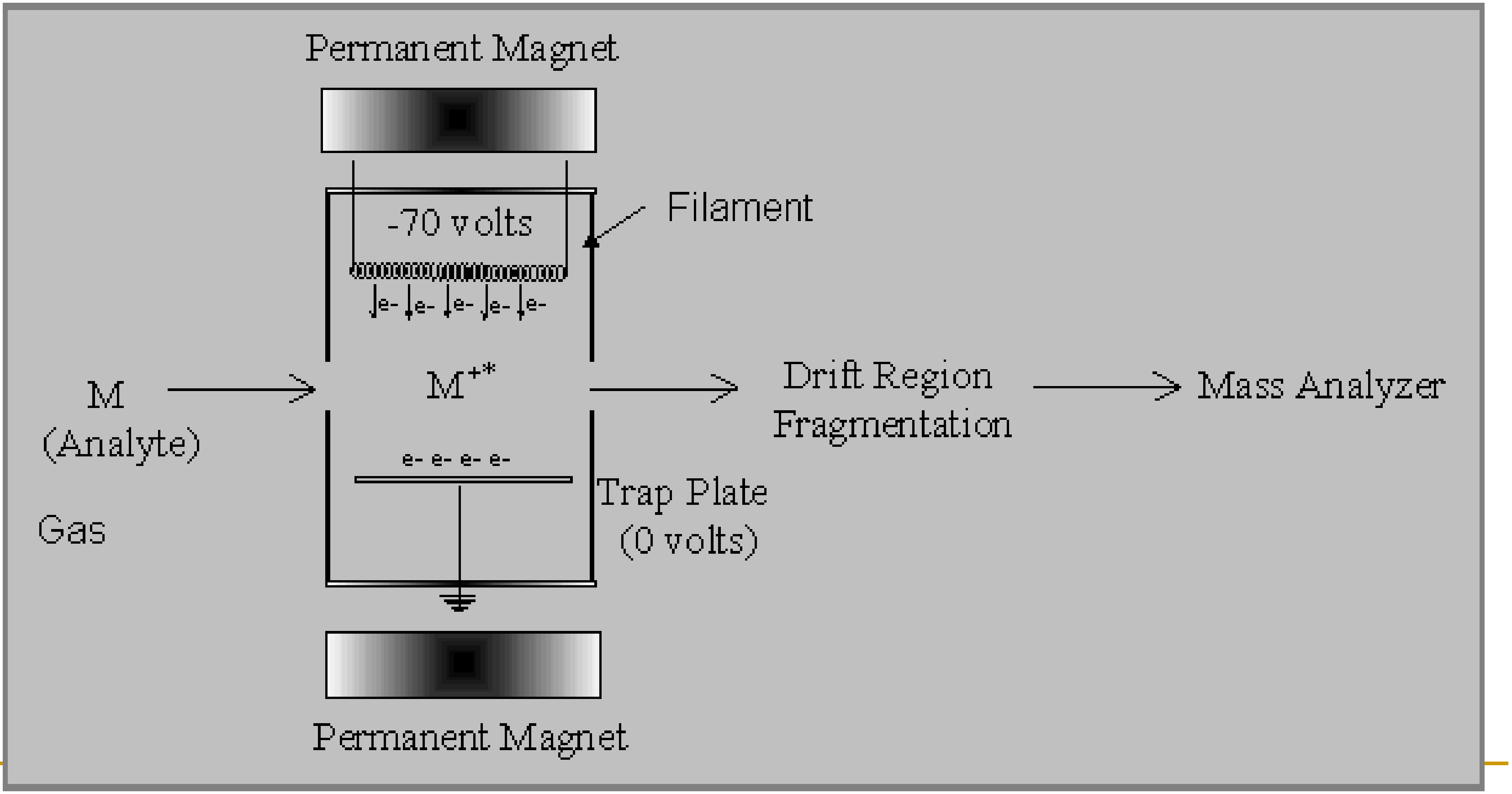
Advantages

- Well-Established
- Fragmentation Libraries
- No Supression
- Insoluble Samples
- Interface to GC
- Non-Polar Samples

Disadvantages

- Parent Identification
- Need Volatile Sample
- Need Thermal Stability
- No Interface to LC
- Low Mass Compounds (<1000 amu)
- Solids Probe Requires Skilled Operator

Electron Impact



http://www.noble.org/PlantBio/MS/ion_tech_main.html

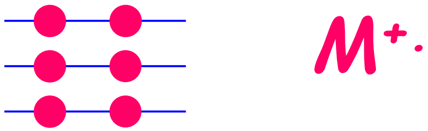
How does ionization occur?

Consider the Ionization potential

- a minimum amount of energy for ion formation to occur.

- the first ionization potential

the energy input required to remove an electron from the highest occupied atomic or molecular orbital of the neutral particle



- **First ionization potential**
  - **in the *5-15 eV* range for most elements**
  - **in the *8-12 eV* range for most organic molecules and radicals**

1 eV=1.6021 $\times 10^{-19}$ Joules=3.8291 $\times 10^{-20}$ calories

- **To remove a second, third, *etc* electron, additional energy is needed.**

- **When excess energy is available, fragmentation of the molecule may also occur during the process of ionization**

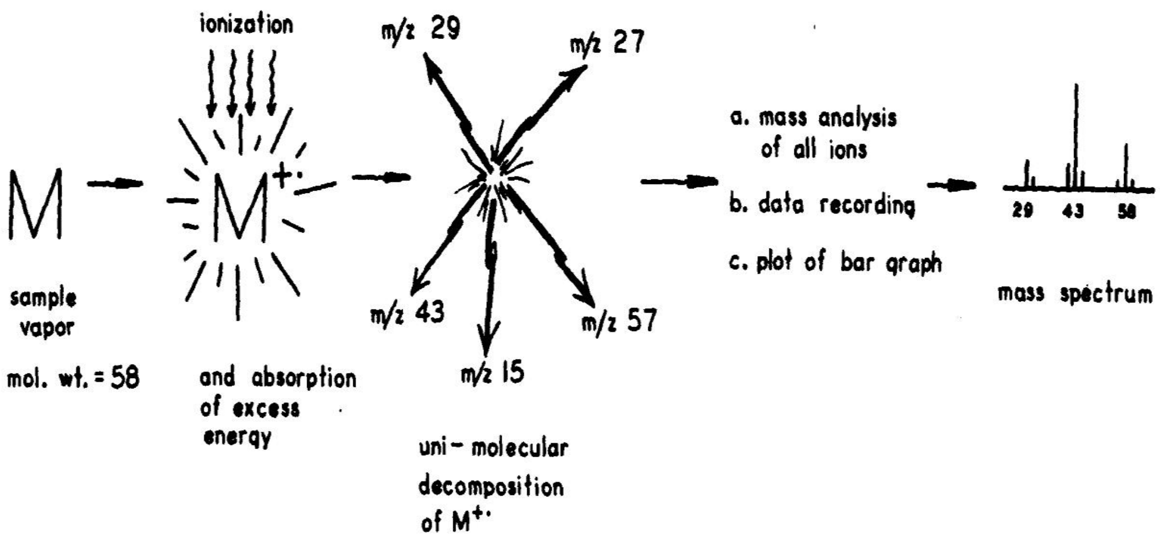


FIG. 1.1. General conceptual scheme for vapor-phase analysis by mass spectrometry.

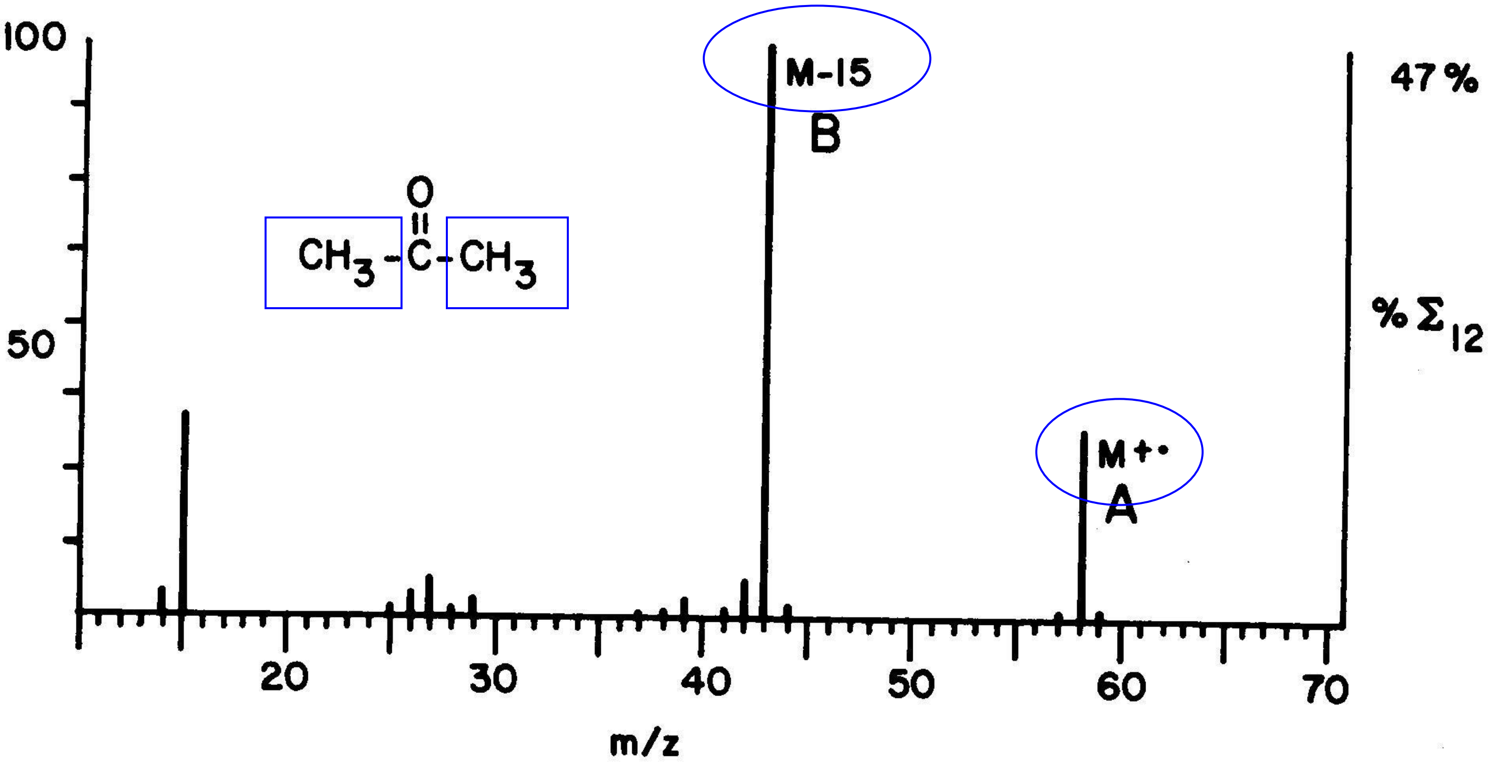
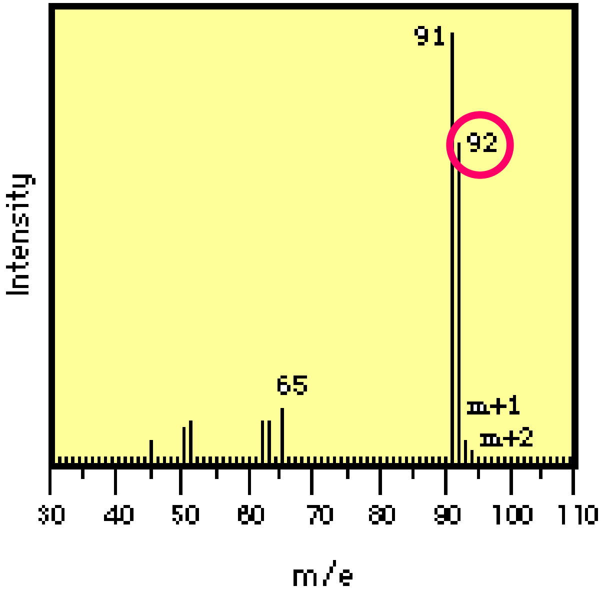


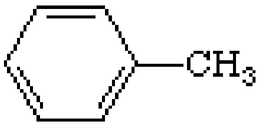
FIG. 1.6. Bar-graph format for the mass spectrum of acetone.

Molecular Ion

- The molecular ion results from ionization of the analyte molecule.
- The molecular ion peak appears at an m/z value numerically equal to the nominal molecular weight (MW) of the compound.
  - The nominal molecular weight is calculated by summation of the atomic masses of the lightest isotope of each element composing the molecule.

Molecular ion ⟶ $MW=M^{+\cdot}$

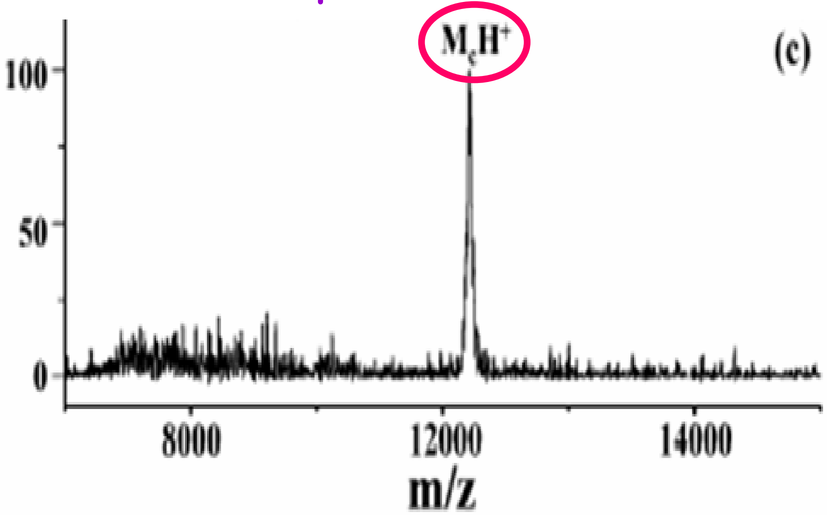




Toluene

$MH^{+}=MW+1$

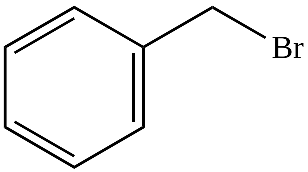
called pseudomolecular ion



Identifying the Molecular Ions

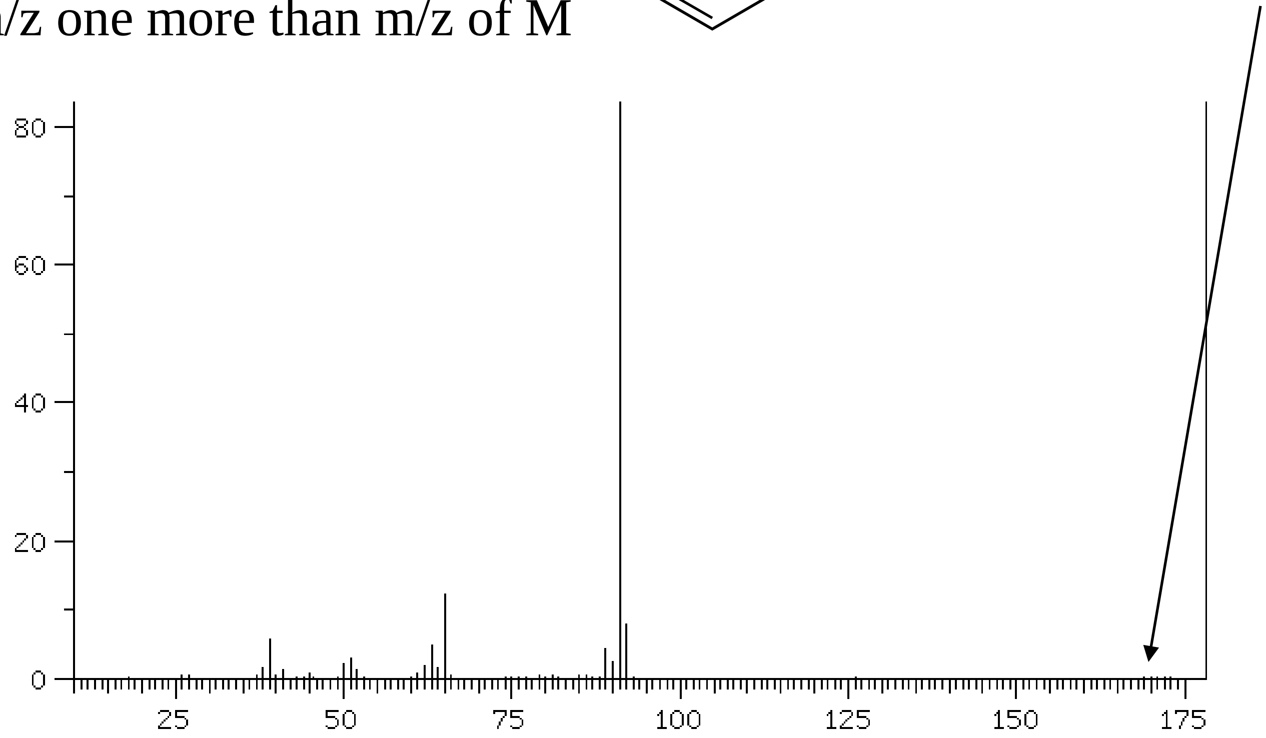
Which peaks are molecular ions?

- Highest m/z not always M
- M+1 has m/z one more than m/z of M



C_7H_7Br

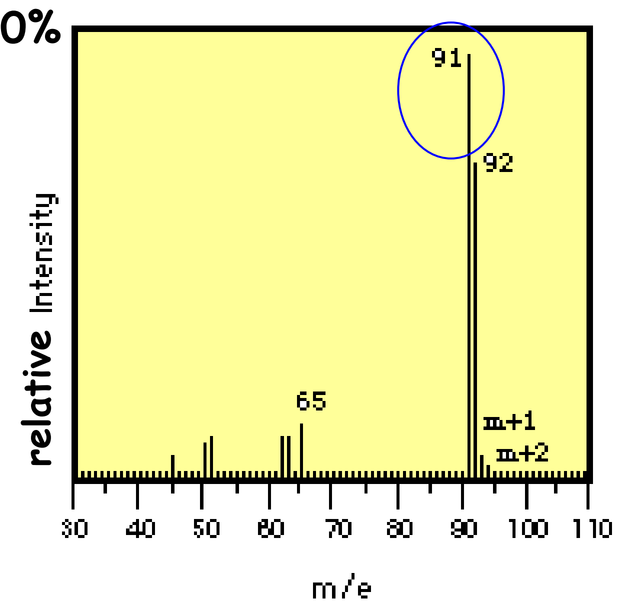
M: m/z = 170

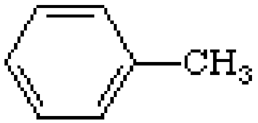


Base peak

- The base peak is the most intense peak in the mass spectrum.
- It is used as the base against which the intensities of all other peaks are normalized.

Where is the base peak?

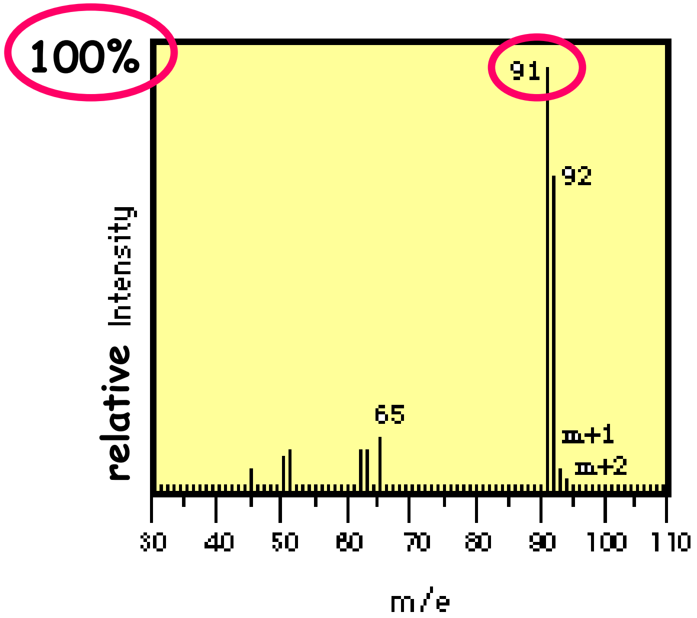


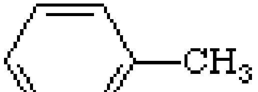


Toluene

Relative Intensity

- The relative intensity of a given peak expresses its intensity **relative to that of the base peak**, the most intense peak in the mass spectrum.





Toluene

Isotopes

+Most elements have more than one stable isotope.

For example, most carbon atoms have a mass of 12 Da, but in nature, 1.1% of C atoms have an extra neutron, making their mass 13 Da.

+Why do we care?

Mass spectrometers can “see” isotope peaks if their resolution is high enough.

If an MS instrument has resolution high enough to resolve these isotopes, better mass accuracy is achieved.

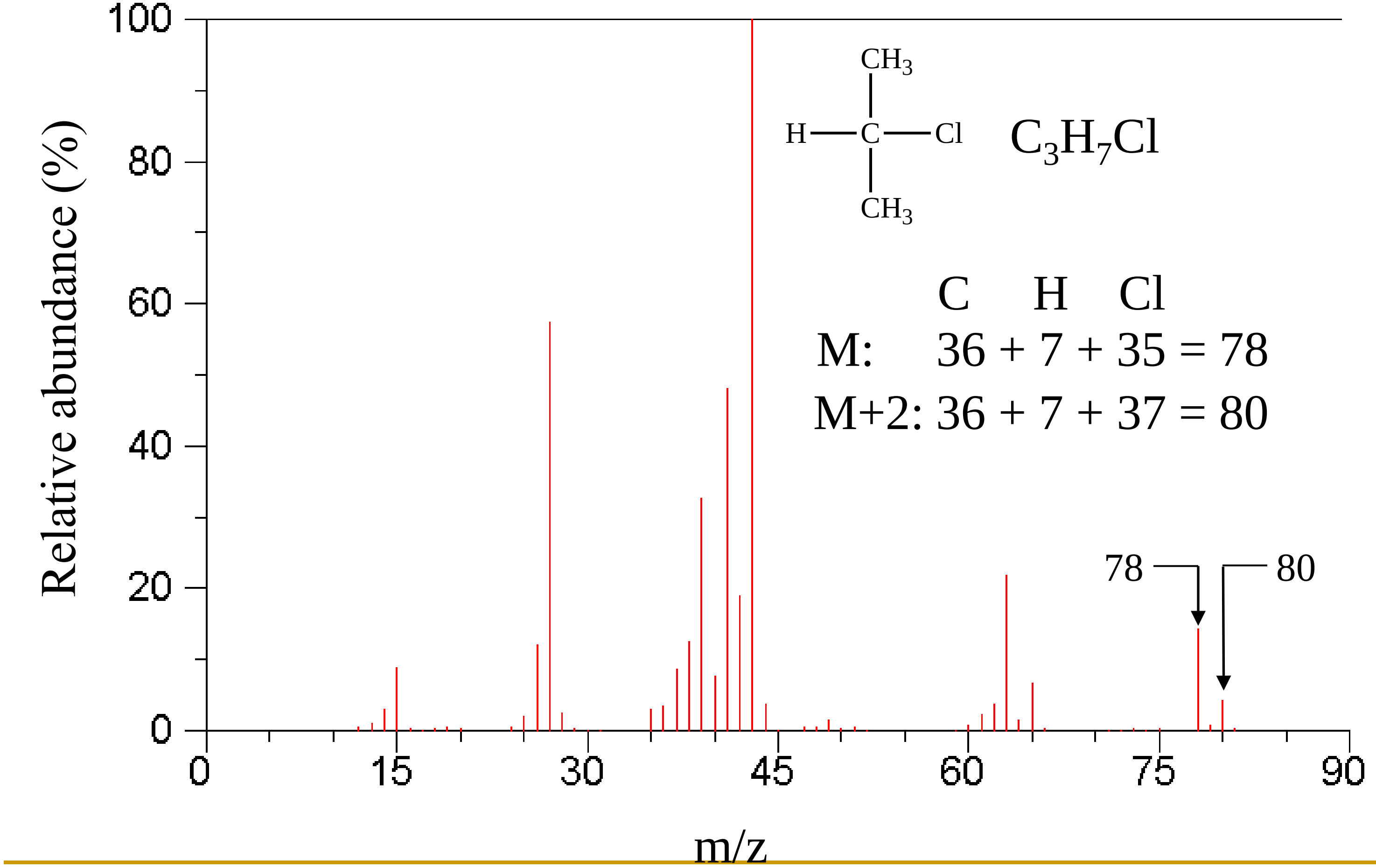
The Mass Spectrum

Origin of Relative Ion Abundances

M contributors		M+1 contributors		M+2 contributors	
Isotope	**Natural Abundance**	**Isotope**	**Natural Abundance**	**Isotope**	**Natural Abundance**
^{1}H	99.9855%	^{2}H	0.015%	^{3}H	ppm
^{12}C	98.893	^{13}C	1.107	^{14}C	ppm
^{14}N	99.634	^{15}N	0.366		
^{16}O	99.759	^{17}O	0.037	^{18}O	0.204
^{19}F	100.0				
^{32}S	95.0	^{33}S	0.76	^{34}S	4.22
^{35}Cl	75.77			^{37}Cl	24.23
^{79}Br	50.69			^{81}Br	49.31
^{127}I	100.0				

Formula from Mass Spectrum

M+2 Contributors



C_3H_7Cl

C H Cl

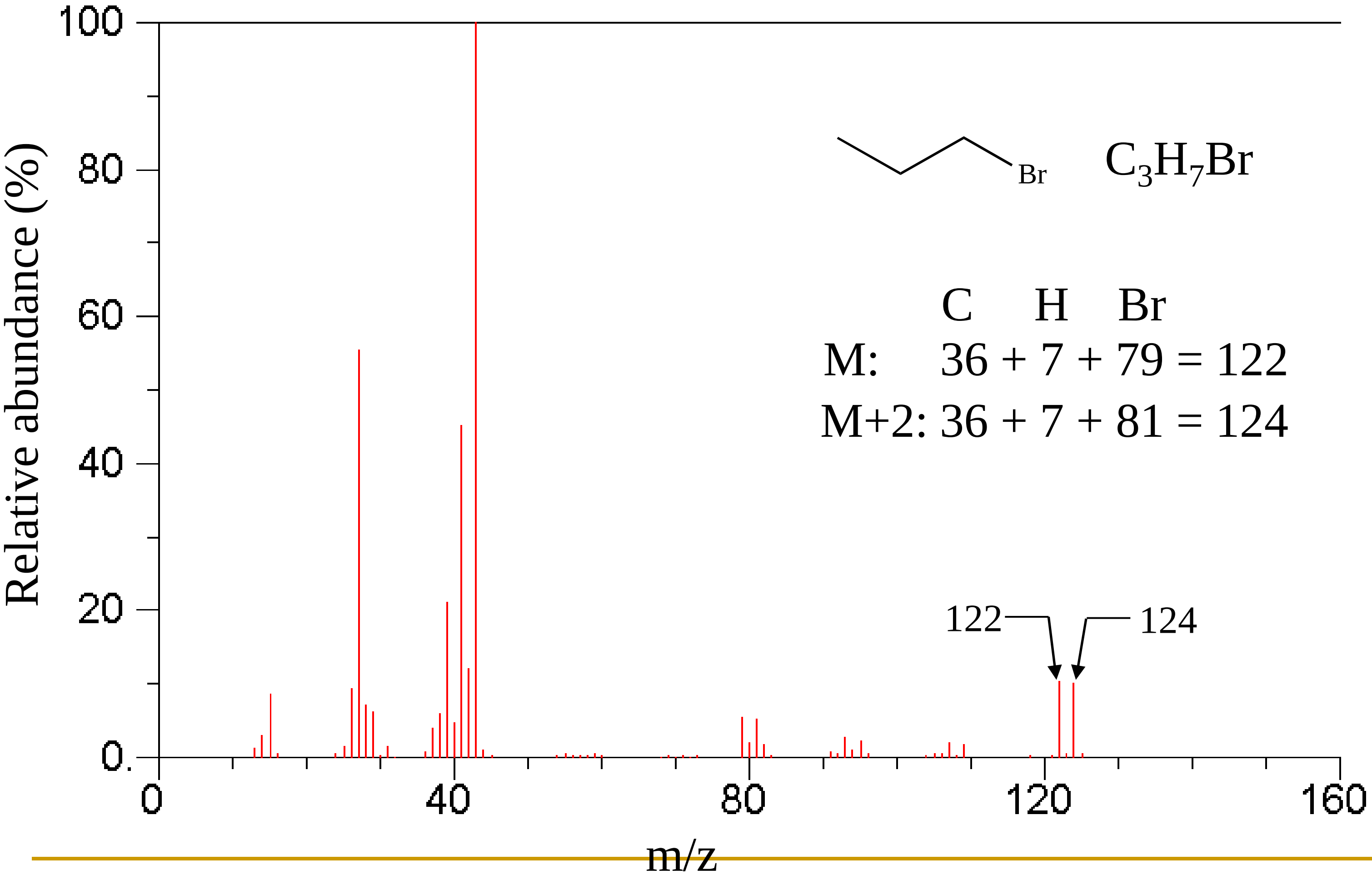
M: 36 + 7 + 35 = 78

M+2: 36 + 7 + 37 = 80

M:M+2
abundance
~3:1

Formula from Mass Spectrum

M+2 Contributors

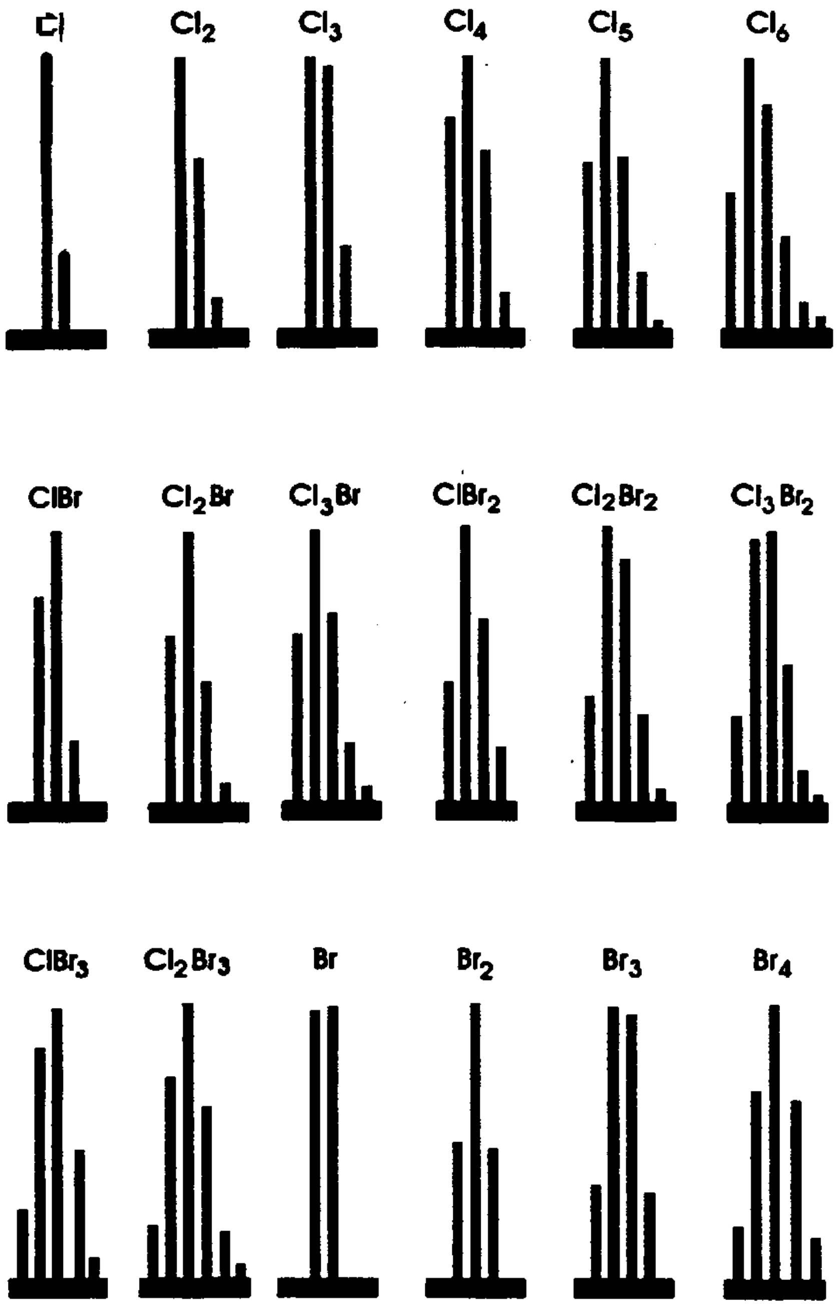


C_3H_7Br

	C	H	Br	
M:	36 +	7 +	79 =	122
M+2:	36 +	7 +	81 =	124

M:M+2
abundance
~1:1

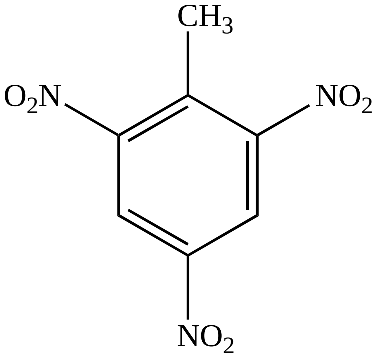
Halogen Isotope Clusters

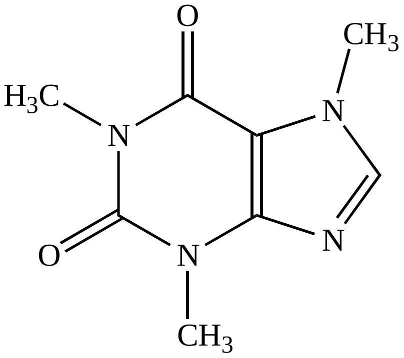


How Many Nitrogen Atoms?

Consider these molecules:

NH_3 H_2NNH_2





Formula:	NH_3	N_2H_4	$C_7H_5N_3O_6$	$C_8H_{10}N_4O_2$
m/z (M):	17	32	227	194

Conclusion

- When m/z (M) = even, number of N in formula is even
- When m/z (M) = odd, number of N in formula is odd

} **The Nitrogen Rule**

How Many Nitrogen Atoms?

A Nitrogen Rule Example

Example: Formula choices from previous mass spectrum

M: m/z = 78 ← m/z even

$C_2H_6O_3$ ← even nitrogen count

C_3H_7Cl ← even nitrogen count

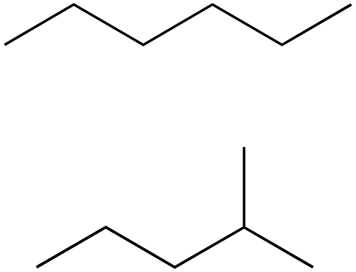
discarded ~~C_5H_4N~~ ← odd nitrogen count

C_6H_6 ← even nitrogen count

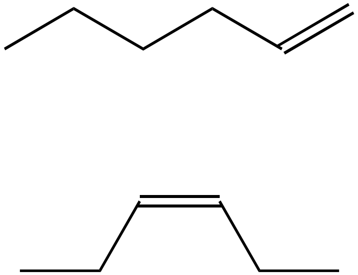
How Many Hydrogen Atoms?

One pi bond

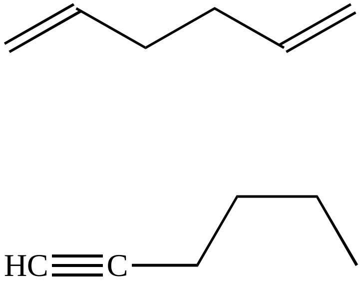
Two pi bonds



C_6H_{14}
max H for 6 C



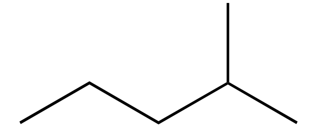
C_6H_{12}
H count = max - 2



C_6H_{10}
H count = max - 4

Conclusion: Each pi bond reduces max hydrogen count by two

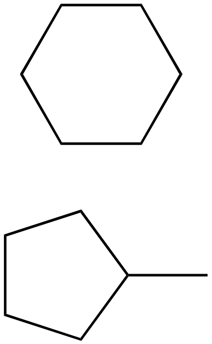
How Many Hydrogen Atoms?



C_6H_{14}

max H for 6 C

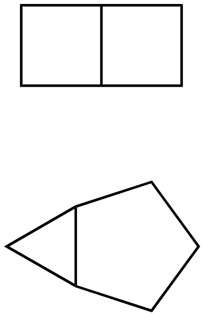
One ring



C_6H_{12}

H count = max - 2

Two rings

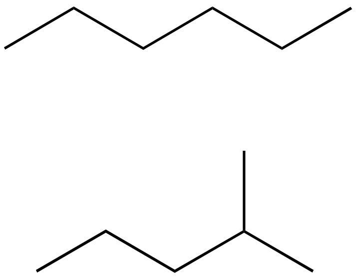


C_6H_{10}

H count = max - 4

Conclusion: Each ring reduces max hydrogen count by two

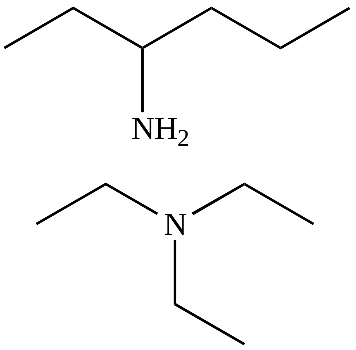
How Many Hydrogen Atoms?



C_6H_{14}

max H for 6 C

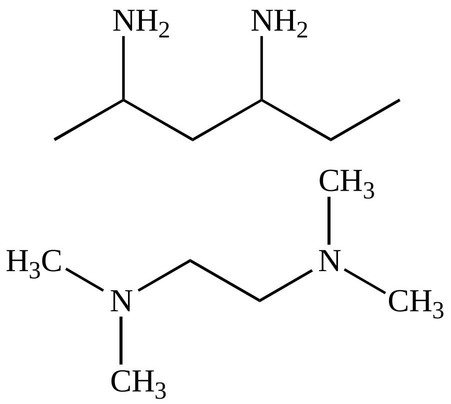
One nitrogen



$C_6H_{15}N$

H count = max + 1

Two nitrogens



$C_6H_{16}N_2$

H count = max + 2

Conclusion:

- Each nitrogen increases max H count by one
- For C carbons and N nitrogens, max number of H = 2C + N + 2

The Hydrogen Rule